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(57) Abstract

The present invention relates to a nucleic acid molecule encoding a $GABA_B$ receptor, or a functionally equivalent modified form thereof, said receptor being selected from the group consisting of human and canine $GABA_B$ receptors.

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NEW NUCLEOTIDE SEQUENCES

TECHNICAL FIELD

The present invention relates in particular to nucleic acid molecules encoding $GABA_B$ receptors, and to methods for screening for compounds which are inhibitors of transient lower esophageal sphincter relaxations (TLESR), said methods comprising the use of a nucleic acid molecule encoding a $GABA_B$ receptor.

BACKGROUND ART

$GABA_B$ receptors

GABA (4-aminobutanoic acid) is an endogenous neurotransmitter in the central and peripheral nervous systems. Receptors for GABA have traditionally been divided into $GABA_A$ and $GABA_B$ receptor subtypes. $GABA_B$ receptors (for a review see Kerr, D.I.B. and Ong, J. (1995) Pharmac. Ther. vol. 67, pp.187-246) belong to the superfamily of G-protein coupled receptors. $GABA_B$ receptor agonists are described as being of use in the treatment of CNS disorders, such as muscle relaxation in spinal spasticity, cardiovascular disorders, asthma, gut motility disorders such as irritable bowel syndrome and as prokinetic and anti-tussive agents. $GABA_B$ receptor agonists have also been disclosed as useful in the treatment of emesis (WO 96/11680).

The cloning of the rat $GABA_B$ receptors $GABA_BR1a$ (SEQ ID NOS: 44 and 45) and $GABA_BR1b$ (SEQ ID NOS: 46 and 47) is disclosed by Kaupmann et al. (1997) Nature, vol. 386, 239-246. The mature rat $GABA_BR1b$ differed from $GABA_BR1a$ in that the N-terminal 147 residues were replaced by 18 different residues. It was presumed that the rat $GABA_BR1a$ and -b receptor variants are derived from the same gene by alternative splicing.

The cloning of the human $GABA_B$ receptor $GABA_BR1b$ is disclosed in WO 97/46675.

Reflux

In some humans, the lower esophageal sphincter (LES) is prone to relaxing more frequently than in other humans. As a consequence, fluid from the stomach can pass into the esophagus since the mechanical barrier is temporarily lost at such times, an event hereinafter referred to as "reflux".

Gastro-esophageal reflux disease (GERD) is the most prevalent upper gastrointestinal tract disease. Current therapy has aimed at reducing gastric acid secretion, or by reducing esophageal acid exposure by enhancing esophageal clearance, lower esophageal sphincter tone and gastric emptying. The major mechanism behind reflux has been considered to depend on a hypotonic lower esophageal sphincter. However, recent research (e.g. Holloway & Dent (1990) Gastroenterol. Clin. N. Amer. 19, 517-535) has shown that most reflux episodes occur during transient lower esophageal sphincter relaxations (TLESR), i.e. relaxations not triggered by swallows. It has also been shown that gastric acid secretion usually is normal in patients with GERD. Consequently, there is a need for compounds which reduce the incidence of TLESR and thereby prevent reflux.

DISCLOSURE OF THE INVENTION

In the applicants' earlier patent application WO 98/11885, filed on 15 September 1997, it is disclosed that $GABA_B$ receptor agonists can be used to reduce the incidence of transient lower esophageal sphincter relaxations (TLESR).

The present invention provides nucleic acid molecules encoding human and canine $GABA_B$ receptors. These nucleic acid molecules will make possible the screening for compounds which are agonists or antagonists of $GABA_B$ receptors, e.g. compounds which are inhibitors of transient lower esophageal sphincter relaxations (TLESR).

Consequently, in a first aspect, the present invention provides a nucleic acid molecule encoding a $GABA_B$ receptor, or a functionally equivalent modified form thereof, said receptor being selected from the group consisting of human and canine $GABA_B$ receptors.

In preferred forms of the invention, the said nucleic acid molecule encodes the human $GABA_B$ receptor 1a (SEQ ID NOS: 48 and 49), 1b (SEQ ID NOS: 50 and 51), 1c (SEQ ID NOS: 54 and 55) or 1d (SEQ ID NOS: 56 and 57); or the canine $GABA_B$ receptor 1a (SEQ ID NOS: 52 and 53) or 1c (SEQ ID NOS: 58 and 59). Accordingly, the invention furthermore provides a nucleic acid molecule selected from:

(a) nucleic acid molecules comprising a nucleotide sequence set forth as SEQ ID NO: 48, 50, 52, 54, 56 or 58;

(b) nucleic acid molecules comprising a nucleotide sequence capable of hybridizing, under stringent hybridization conditions, to a nucleotide sequence complementary to the polypeptide coding region of a DNA molecule as defined in (a); and

(c) nucleic acid molecules comprising a nucleotide sequence which is degenerate as a result of the genetic code to a nucleotide sequence as defined in (a) or (b).

Furthermore, the invention provides nucleic acid molecules of genomic origin encoding human $GABA_B$ receptors (SEQ ID NOS: 60 and 61) as well as nucleic acid molecules (set forth as SEQ ID NO: 70, 72, 74, 76, 78, 80, 82, 84) encoding additional isoforms of the human $GABA_B$ receptor which isoforms can be shown to be generated by alternative splicing.

It should thus be understood that the nucleic acid molecule according to the invention is not to be limited strictly to molecules comprising the sequences set forth as SEQ ID : 48, 50, 52, 54, 56 or 58. Rather the invention encompasses nucleic acid molecules carrying modifications like substitutions, small deletions, insertions or inversions, which nevertheless encode proteins having substantially the biochemical activity of the $GABA_B$ receptors according to the invention. Included in the invention are consequently nucleic acid molecules, the nucleotide sequence of which is at least 95% homologous, preferably at least 96%, 97%, 98% or 99% homologous, with the nucleotide sequence shown as SEQ ID NO: 48, 50, 52, 54, 56 or 58 in the Sequence Listing.

The term "stringent hybridization conditions" is known in the art from standard protocols (e.g. Current Protocols in Molecular Biology, editors F. Ausubel et al., John Wiley and Sons, Inc. 1994) and could be understood as as stringent or more stringent than those defined by e.g. hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at +65°C, and washing in 0.1xSSC / 0.1% SDS at +68°C.

Included in the invention is also a nucleic acid molecule which nucleotide sequence is degenerate, because of the genetic code, to a nucleic acid of the present invention and more particularly to one of the nucleotide sequences set forth as SEQ ID NOs: 48, 50, 52, 54, 56 and 58. A sequential grouping of three nucleotides, a "codon", codes for one amino acid. Since there are 64 possible codons, but only 20 natural amino acids, most amino acids are coded for by more than one codon. This natural "degeneracy", or "redundancy", of the genetic code is well known in the art. It will thus be appreciated that the nucleic acid sequences shown in the Sequence Listing is only an example within a large but definite group of nucleic acid sequences which will encode the polypeptide as described above.

In a further aspect, the invention provides a recombinant polypeptide encoded by a nucleotide sequence of the present invention, encoding a $GABA_B$ receptor. In preferred forms of the inventions, the said polypeptide comprises an amino acid sequence set forth as SEQ ID NO: 49, 51, 53, 55, 57, 59, 71, 73, 75, 77, 79, 81, 83 or 85 in the Sequence Listing. However, polypeptides of the present invention are not to limited to those having an amino acid sequence identical with one of SEQ ID NOs: 49, 51, 53, 55, 59, 71, 73, 75, 77, 79, 81, 83 or 85 in the Sequence Listing. Rather the invention encompasses polypeptides carrying modifications such as substitutions, small deletions, insertions or inversions, which polypeptides nevertheless have substantially the biological activities of the $GABA_B$ receptor. Included in the invention are consequently polypeptides, the amino acid sequence of which is at least 95% homologous, preferably at least 96%, 97%, 98% or 99% homologous, with one of the amino acid sequences described by SEQ ID NOs: 49, 51, 53, 55, 57 59, 71, 73, 75, 77, 79, 81, 83 and 85 in the Sequence Listing.

Included in the invention are polypeptides of the present invention which have been post-translationally modified, e.g. by cleavage of an N-terminal signal sequence which can be e.g. 1 to 25 amino acids long.

In yet another aspect, the invention provides a vector transformed with a nucleic acid molecule of the present invention. The said vector can e.g. be a replicable expression vector which carries and is capable of mediating the expression of a nucleic acid molecule according to the invention. In the present context the term "replicable" means that the vector is able to replicate in a given type of host cell into which is has been introduced. Examples of vectors are viruses such as bacteriophages, cosmids, plasmids and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known in the art.

Included in the invention is also a cultured host cell harbouring a vector according to the invention. Such a host cell can be a prokaryotic cell, a unicellular eukaryotic cell or a cell derived from a multicellular organism. The host cell can thus e.g. be a bacterial cell such as an *E. coli* cell; a cell from a yeast such as *Saccharomyces cervisiae* or *Pichia pastoris*, or a mammalian cell. The methods employed to effect introduction of the vector into the host cell are standard methods well-known to a person familiar with recombinant DNA methods.

A further aspect of the invention is a process for production of a $GABA_B$ receptor polypeptide according to the invention, said process comprising culturing a host cell as defined above under conditions whereby the said polypeptide is produced, and recovering the said polypeptide.

A further important aspect of the invention is a method for the screening of compounds which are inhibitors of transient lower esophageal sphincter relaxations (TLESR), said method comprising the use of a nucleic acid molecule encoding a $GABA_B$ receptor. The said nucleic acid molecule encoding a $GABA_B$ receptor can e.g. be one of the nucleic acid molecules according to the invention encoding human or canine $GABA_B$ receptors. However, it should be understood that this aspect of the invention is not limited to the use

of the said human and canine $GABA_B$ receptors, but rather encompasses the use of any $GABA_B$ receptor for screening for compounds which are inhibitors of transient lower esophageal sphincter relaxations.

In yet another important aspect, the invention provides a method for the screening of compounds which are agonists or antagonists to a $GABA_B$ receptor, said method comprising the use of a nucleic acid molecule, according to the invention, encoding human or canine $GABA_B$ receptors.

Brief Description of the Drawings

Figure 1: Map of the human $GABA_B$ receptor gene.

The exon/intron organisation is shown. Exons are indicated as solid boxes numbered 1-23. The part of intron 5 that is retained together with exon 6 giving rise to $GABA_B$ receptor 1b is indicated as an open box.

Figure 2: Expression of human $GABA_B$ receptor 1b isoform in transfected C127 cells.

Western blot analysis of transfected C127 cells using a polyclonal anti-human $GABA_B$ receptor antibody. Lane 1: Untransfected C127 whole cell lysate. Lanes 2-7: Whole cell lysates of six independent clones transfected with human $GABA_B$ receptor 1b isoform encoding cDNA. The clones analysed in lanes 4 to 7 express a $GABA_B$ receptor of expected molecular weight (arrow).

Figure 3: Expression of human $GABA_B$ receptor 1d isoform in transfected C127 cells.

Western blot analysis of transfected C127 cells using a polyclonal anti-human $GABA_B$ receptor antibody. Lanes 1-3: Concentrated culture media from three independent C127 clones transfected with a cDNA expression construct encoding the human $GABA_B$ receptor

1d isoform. Lanes 4-6: Whole cell lysates corresponding to the clones analysed in lanes 1-3. The experiment revealed that the human $GABA_B$ receptor 1d cDNA encodes a secreted isoform. The arrow indicates the bands corresponding to the 1d isoform.

Figure 4: Expression of human $GABA_B$ receptor 1d isoform in *E. coli*.

Western blot analysis of transformed *E. coli* cells using a polyclonal anti-human $GABA_B$ receptor antibody. Lane 1: Lysate from an uninduced *E. coli* culture transformed with an pET-based expression construct encoding the human $GABA_B$ receptor 1d cDNA. Lane 2: Lysate from an IPTG-induced *E. coli* culture transformed with an expression construct encoding the human $GABA_B$ receptor 1d cDNA. Lane 3: Lysate from an IPTG-induced *E. coli* culture transformed with an expression construct encoding an unrelated protein. Lane 4: The BSA-conjugated peptide previously used for immunization was loaded on the gel as a positive antibody control.

The screening methods according to the invention can e.g. comprise the steps (a) transforming a cultured cell with a nucleic acid molecule encoding a $GABA_B$ receptor, so that a $GABA_B$ receptor is expressed on the surface of the cell; (b) contacting a test compound with the said cell; and (c) determining whether the test compound binds to, and/or activates, the $GABA_B$ receptor.

In particular $GABA_B$ receptor expressing cells, transgenic animals or cells and tissues derived thereof, nay be used to screen substance libraries for antagonist or agonist activities. For this purpose, $GABA_B$ receptor expression may be directed to cells and tissues containing, either naturally or artificially, the necessary components allowing correct receptor transport and processing as well as coupling to second messenger pathways. Screening may be performed as ligand binding assays or functional assays. For screening, cells and tissues my be prepared in various ways, each uniquely suited to its purpose. Ligand binding assays are performed *in vivo* or *in vitro* using e.g. radiolabelled GABA. Functional assays examplified by, but not limited to, Ca^{++}-responses, cAMP-

FIGURE 1A

-243	TGACCTCGGGGCAGGTCCTGGTGCAGAGCGTCGCCAAGGACGCCGAGAGGGAGGCGGGAT	-184
-183	TGCCCAGACATCCTTCAGCGAAGTGCATGTGTGTTTGTAAACCATCGTTGGCTGTCGGGA	-124
-123	GACCGCGAGGACCGGTCCAGGCTGCGGCGGAGTCGAGGGCGAGGGAGAGGCCGCGTGAGT	-64
-63	GAGCAGAGTCCAGAGCCGTGCGCCCCCAGAACTGCGCGTCCGCCCCGTGCACCCCCGCGC	-4
-3	GCCATGCCCAGTTGCCCCGCGCGCTCTGCTACGGGCCCGCTCTCCATCATGGGCCTCATG	57
58	CCGCTCACCAAGGAGGTGGCCAAGGGCAGCATCGGGCGCGGTGTGCTCCCCGCCGTGGAA	117
118	CTGGCCATCGAGCAGATCCGCAACGAGTCACTCCTGCGCCCCTACTTCCTCGACCTGCGG	177
178	CTCTATGACACGGAGTGCGACAACGCAAAAGGGTTGAAAGCCTTCTACGATGCGATAAAA	237
238	TACGGGCCGAACCACTTGATGGTGTTTGGAGGCGTCTGTCCATCCGTCACATCCATCATT	297
298	GCAGAGTCCCTCCAAGGCTGGAATCTGGTGCAGCTTTCTTTTGCTGCAACCACGCCTGTT	357
358	CTAGCCGATAAGAAAAAATACCCTTATTTCTTTCGGACCGTCCCATCAGACAATGCGGTG	417
418	AATCCAGCCATTCTGAAGTTGCTCAAGCACTACCAGTGGAAGCGCGTGGGCACGCTGACG	477
478	CAAGACGTTCAGAGGTTCTCTGAGGTGCGGAATGACCTGACTGGAGTTCTGTATGGCGAG	537

FIGURE 1B

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 538  GACATTGAGATTTCAGACACCGAGAGCTTCTCCAACGATCCCTGTACCAGTGTCAAAAAG   597
 598  CTGAAGGGGAATGATGTGCGGATCATCCTTGGCCAGTTTGACCAGAATATGGCAGCAAAA   657
 658  GTGTTCTGTTGTGCATACGAGGAGAACATGTATGGTAGTAAATATCAGTGGATCATTCCG   717
 718  GGCTGGTACGAGCCTTCTTGGTGGGAGCAGGTGCACACGGAAGCCAACTCATCCCGCTGC   777
 778  CTCCGGAAGAATCTGCTTGCTGCCATGGAGGGCTACATTGGCGTGGATTTCGAGCCCCTG   837
 838  AGCTCCAAGCAGATCAAGACCATCTCAGGAAAGACTCCACAGCAGTATGAGAGAGAGTAC   897
 898  AACAACAAGCGGTCAGGCGTGGGGCCCAGCAAGTTCCACGGGTACGCCTACGATGGCATC   957
 958  TGGGTCATCGCCAAGACACTGCAGAGGGCCATGGAGACACTGCATGCCAGCAGCCGGCAC  1017
1018  CAGCGGATCCAGGACTTCAACTACACGGACCACACGCTGGGCAGGATCATCCTCAATGCC  1077
1078  ATGAACGAGACCAACTTCTTCGGGGTCACGGGTCAAGTTGTATTCCGGAATGGGGAGAGA  1137
1138  ATGGGGACCATTAAATTTACTCAATTTCAAGACAGCAGGGAGGTGAAGGTGGGAGAGTAC  1197
1198  AACGCTGTGGCCGACACACTGGAGATCATCAATGACACCATCAGGTTCCAAGGATCCGAA  1257
1258  CCACCAAAAGACAAGACCATCATCCTGGAGCAGCTGCGGAAGATCTCCCTACCTCTCTAC  1317
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FIGURE 1C

1318	AGCATCCTCTCTGCCCTCACCATCCTCGGGATGATCATGGCCAGTGCTTTTCTCTTCTTC	1377
1378	AACATCAAGAACCGGAATCAGAAGCTCATAAAGATGTCGAGTCCATACATGAACAACCTT	1437
1438	ATCATCCTTGGAGGGATGCTTTCCTATGCTTCCATATTTCTCTTTGGCCTTGATGGATCC	1497
1498	TTTGTCTCTGAAAAGACCTTTGAAACACTTTGCACCGTCAGGACCTGGATTCTCACCGTG	1557
1558	GGCTACACGACCGCTTTTGGGGCCATGTTTGCAAAGACCTGGAGAGTCCACGCCATCTTC	1617
1618	AAAAATGTGAAAATGAAGAAGAAGATCATCAAGGACCAGAAACTGCTTGTGATCGTGGGG	1677
1678	GGCATGCTGCTGATCGACCTGTGTATCCTGATCTGCTGGCAGGCTGTGGACCCCCTGCGA	1737
1738	AGGACAGTGGAGAAGTACAGCATGGAGCCGGACCCAGCAGGACGGGATATCTCCATCCGC	1797
1798	CCTCTCCTGGAGCACTGTGAGAACACCCATATGACCATCTGGCTTGGCATCGTCTATGCC	1857
1858	TACAAGGGACTTCTCATGTTGTTCGGTTGTTTCTTAGCTTGGGAGACCCGCAACGTCAGC	1917
1918	ATCCCCGCACTCAACGACAGCAAGTACATCGGGATGAGTGTCTACAACGTGGGGATCATG	1977
1978	TGCATCATCGGGGCCGCTGTCTCCTTCCTGACCCGGGACCAGCCCAATGTGCAGTTCTGC	2037
2038	ATCGTGGCTCTGGTCATCATCTTCTGCAGCACCATCACCCTCTGCCTGGTATTCGTGCCG	2097

FIGURE 1D

2098	AAGCTCATCACCCTGAGAACAAACCCAGATGCAGCAACGCAGAACAGGCGATTCCAGTTC	2157
2158	ACTCAGAATCAGAAGAAAGAAGATTCTAAAACGTCCACCTCGGTCACCAGTGTGAACCAA	2217
2218	GCCAGCACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGAATGAAGATC	2277
2278	ACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACACACCAGAAAAG	2337
2338	ACCACCTACATTAAACAGAACCACTACCAAGAGCTCAATGACATCCTCAACCTGGGAAAC	2397
2398	TTCACTGAGAGCACAGATGGAGGAAAGGCCATTTTAAAAAATCACCTCGATCAAAATCCC	2457
2458	CAGCTACAGTGGAACACAACAGAGCCCTCTCGAACATGCAAAGATCCTATAGAAGATATA	2517
2518	AACTCTCCAGAACACATCCAGCGTCGGCTGTCCCTCCAGCTCCCCATCCTCCACCACGCC	2577
2578	TACCTCCCATCCATCGGAGGCGTGGACGCCAGCTGTGTCAGCCCCTGCGTCAGCCCCACC	2637
2638	GCCAGCCCCCGCCACAGACATGTGCCACCCTCCTTCCGAGTCATGGTCTCGGGCCTGTAA	2697
2698	GGGTGGGAGGCCTGGGCCCGGGGCCTCCCCCGTGACAGAACCACACTGGGCAGAGGGGTC	2757
2758	TGCTGCAGAAACACTGTCGGCTCTGGCTGCGGAGAAGCTGGGCACCATGGCTGGCCTCTC	2817
2818	AGGACCACTCGGATGGCACTCAGGTGGACAGGACGGGGCAGGGGGAGACTTGGCACCTGA	2877

FIGURE 1E

2878	CCTCGAGCCTTATTTGTGAAGTCCTTATTTCTTCACAAAGAAGAGGAACGGAAATGGGAC	2937
2938	GTCTTCCTTAACATCTGCAAACAAGGAGGCGCTGGGATATCAAACTTGCAAAAAAAAAAA	2997
2998	AAAA	3001

FIGURE 2A

1	M	P	S	C	P	A	R	S	A	T	G	P	L	S	I	M	G	L	M	P	20
21	L	T	K	E	V	A	K	G	S	I	G	R	G	V	L	P	A	V	E	L	40
41	A	I	E	Q	I	R	N	E	S	L	L	R	P	Y	F	L	D	L	R	L	60
61	Y	D	T	E	C	D	N	A	K	G	L	K	A	F	Y	D	A	I	K	Y	80
81	G	P	N	H	L	M	V	F	G	G	V	C	P	S	V	T	S	I	I	A	100
101	E	S	L	Q	G	W	N	L	V	Q	L	S	F	A	A	T	T	P	V	L	120
121	A	D	K	K	K	Y	P	Y	F	F	R	T	V	P	S	D	N	A	V	N	140
141	P	A	I	L	K	L	L	K	H	Y	Q	W	K	R	V	G	T	L	T	Q	160
161	D	V	Q	R	F	S	E	V	R	N	D	L	T	G	V	L	Y	G	E	D	180
181	I	E	I	S	D	T	E	S	F	S	N	D	P	C	T	S	V	K	K	L	200
201	K	G	N	D	V	R	I	I	L	G	Q	F	D	Q	N	M	A	A	K	V	220
221	F	C	C	A	Y	E	E	N	M	Y	G	S	K	Y	Q	W	I	I	P	G	240

FIGURE 2B

241	W	Y	E	P	S	W	W	E	Q	V	H	T	E	A	N	S	S	R	C	L	260
261	R	K	N	L	L	A	A	M	E	G	Y	I	G	V	D	F	E	P	L	S	280
281	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	E	R	E	Y	N	300
301	N	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	Y	D	G	I	W	320
321	V	I	A	K	T	L	Q	R	A	M	E	T	L	H	A	S	S	R	H	Q	340
341	R	I	Q	D	F	N	Y	T	D	H	T	L	G	R	I	I	L	N	A	M	360
361	N	E	T	N	F	F	G	V	T	G	Q	V	V	F	R	N	G	E	R	M	380
381	G	T	I	K	F	T	Q	F	Q	D	S	R	E	V	K	V	G	E	Y	N	400
401	A	V	A	D	T	L	E	I	I	N	D	T	I	R	F	Q	G	S	E	P	420
421	P	K	D	K	T	I	I	L	E	Q	L	R	K	I	S	L	P	L	Y	S	440
441	I	L	S	A	L	T	I	L	G	M	I	M	A	S	A	F	L	F	F	N	460
461	I	K	N	R	N	Q	K	L	I	K	M	S	S	P	Y	M	N	N	L	I	480

FIGURE 2C

481	I	L	G	G	M	L	S	Y	A	S	I	F	L	F	G	L	D	G	S	F	500
501	V	S	E	K	T	F	E	T	L	C	T	V	R	T	W	I	L	T	V	G	520
521	Y	T	T	A	F	G	A	M	F	A	K	T	W	R	V	H	A	I	F	K	540
541	N	V	K	M	K	K	K	I	I	K	D	Q	K	L	L	V	I	V	G	G	560
561	M	L	L	I	D	L	C	I	L	I	C	W	Q	A	V	D	P	L	R	R	580
581	T	V	E	K	Y	S	M	E	P	D	P	A	G	R	D	I	S	I	R	P	600
601	L	L	E	H	C	E	N	T	H	M	T	I	W	L	G	I	V	Y	A	Y	620
621	K	G	L	L	M	L	F	G	C	F	L	A	W	E	T	R	N	V	S	I	640
641	P	A	L	N	D	S	K	Y	I	G	M	S	V	Y	N	V	G	I	M	C	660
661	I	I	G	A	A	V	S	F	L	T	R	D	Q	P	N	V	Q	F	C	I	680
681	V	A	L	V	I	I	F	C	S	T	I	T	L	C	L	V	F	V	P	K	700
701	L	I	T	L	R	T	N	P	D	A	A	T	Q	N	R	R	F	Q	F	T	720

FIGURE 2D

721	Q	N	Q	K	K	E	D	S	K	T	S	T	S	V	T	S	V	N	Q	A	740
741	S	T	S	R	L	E	G	L	Q	S	E	N	H	R	L	R	M	K	I	T	760
761	E	L	D	K	D	L	E	E	V	T	M	Q	L	Q	D	T	P	E	K	T	780
781	T	Y	I	K	Q	N	H	Y	Q	E	L	N	D	I	L	N	L	G	N	F	800
801	T	E	S	T	D	G	G	K	A	I	L	K	N	H	L	D	Q	N	P	Q	820
821	L	Q	W	N	T	T	E	P	S	R	T	C	K	D	P	I	E	D	I	N	840
841	S	P	E	H	I	Q	R	R	L	S	L	Q	L	P	I	L	H	H	A	Y	860
861	L	P	S	I	G	G	V	D	A	S	C	V	S	P	C	V	S	P	T	A	880
881	S	P	R	H	R	H	V	P	P	S	F	R	V	M	V	S	G	L			898

FIGURE 3A

```
   1  ATGGCTTCCCCGCCGAGCTCCGGGCAGCCCCGGCCGCCGCCGCCGCCGCCGCCCGCG   60
  61  CGCCTGCTGCTGCCCCTGCTGCTGTCGCTGCTGCTGTGGTTGGCGCCCGGGGCCTGGGGC  120
 121  TGGACGCGGGGCGCCCCCCGGCCGCCGCCCAGCAGCCCGCCGCTCTCCATCATGGGCCTC  180
 181  ATGCCGCTCACCAAGGAGGTGGCCAAGGGCAGCATCGGGCGCGGCGTGCTCCCCGCCGTG  240
 241  GAGCTAGCCATCGAGCAGATCCGCAACGAGTCACTCCTGCGCCCCTACTTCCTGGACCTG  300
 301  CGACTCTATGACACCGAGTGTGACAATGCAAAGGGACTGAAAGCCTTCTATGACGCAATA  360
 361  AAGTATGGGCCGAACCATTTGATGGTGTTTGGAGGCGTCTGTCCGTCTGTCACATCTATT  420
 421  ATCGCGGAGTCCCTCCAAGGCTGGAATCTGGTGCAGCTTTCCTTCGCCGCCACCACGCCT  480
 481  GTTCTTGCGGATAAGAAGAAGTACCCGTATTTCTTCCGGACGGTGCCGTCAGACAACGCG  540
 541  GTGAACCCCGCCATCCTGAAGCTCCTGAAGCACTTCCGCTGGCGGCGTGTGGGCACACTC  600
 601  ACGCAGGACGTGCAGCGCTTCTCCGAGGTGAGGAATGACCTGACTGGGGTTCTGTATGGG  660
 661  GAAGATATTGAGATCTCAGACACAGAGAGTTTCTCCAATGATCCCTGCACCAGCGTCAAA  720
 721  AAGCTCAAGGGGAATGACGTGCGGATCATCCTTGGCCAGTTTGACCAGAATATGGCAGCA  780
 781  AAAGTCTTCTGTTGTGCCTTCGAGGAGAGCATGTTTGGCAGCAAGTACCAGTGGATCATC  840
```

FIGURE 3B

841	CCGGGATGGTACGAGCCTGCGTGGTGGGAGCAGGTGCATGTGGAGGCCAATTCCTCACGC	900
901	TGCCTGCGCAGAAGCCTCCTGGCTGCCATGGAAGGTTACATCGGAGTGGACTTTGAGCCC	960
961	CTGAGCTCCAAACAAATCAAGACCATCTCAGGGAAGACTCCACAGCAGTATGAAAGAGAG	1020
1021	TACAACAGCAAACGTTCAGGCGTGGGGCCCAGCAAGTTCCATGGGTACGCCTACGATGGG	1080
1081	ATCTGGGTCATCGCCAAGACCCTACAGAGGGCCATGGAGACACTGCATGCCAGTAGCAGG	1140
1141	CACCAGCGGATCCAGGACTTCAACTACACAGACCACACGCTGGGCAAAATCATCCTCAAT	1200
1201	GCCATGAACGAGACCAACTTCTTCGGGGTCACGGGTCAAGTTGTGTTCCGGAACGGGGAG	1260
1261	AGAATGGGAACCATTAAATTTACTCAATTTCAAGACAGCAGAGAGGTGAAGGTCGGCGAA	1320
1321	TACAACGCGGTGGCTGACACACTGGAGATCATCAATGACACCATAAGGTTCCAGGGGTCC	1380
1381	GAGCCACCCAAGGACAAGACCATCATTCTGGAGCAGCTTCGGAAGATCTCGCTTCCACTG	1440
1441	TATAGCATCCTGTCCGCTCTCACCATCCTCGGCATGATCATGGCCAGCGCCTTCCTCTTC	1500
1501	TTCAACATCAAGAACCGGAACCAAAAGCTGATTAAGATGTCAAGCCCCTACATGAACAAC	1560
1561	CTCATCATCCTGGGAGGAATGCTGTCCTATGCATCCATCTTCCTCTTTGGCCTCGATGGG	1620
1621	TCCTTCGTCTCAGAAAAGACCTTTGAAACACTCTGCACGGTCCGGACCTGGATTCTCACC	1680

FIGURE 3C

1681	GTGGGCTACACAACTGCCTTTGGGGCCATGTTTGCAAAGACCTGGAGGGTCCATGCCATC	1740
1741	TTCAAAAATGTGAAGATGAAGAAGAAGATCATCAAAGACCAGAAGCTGCTTGTGATTGTG	1800
1801	GGGGGCATGCTGCTCATCGACCTGTGCATCCTGATCTGTTGGCAGGCTGTGGACCCCCTG	1860
1861	CGGAGGACAGTAGAGAGGTACAGCATGGAGCCGGACCCAGCAGGCCGGGACATCTCCATC	1920
1921	CGCCCATTGCTGGAACACTGCGAAAACACCCACATGACCATCTGGCTTGGCATTGTCTAC	1980
1981	GCCTACAAGGGGCTCCTCATGCTATTCGGTTGTTTCTTGGCATGGGAAACCCGCAATGTG	2040
2041	AGCATCCCTGCCCTCAACGACAGCAAGTACATCGGCATGAGTGTGTACAATGTGGGGATC	2100
2101	ATGTGCATCATCGGGGCTGCTGTCTCCTTCCTGACGCGTGACCAGCCCAACGTGCAGTTC	2160
2161	TGCATCGTGGCCCTGGTCATCATCTTCTGCAGCACCATCACTCTCTGCCTGGTGTTTGTG	2220
2221	CCAAAGCTCATTACTCTGAGGACAAACCCTGACGCAGCCACTCAGAACAGGCGGTTCCAG	2280
2281	TTCACACAGAACCAGAAGAAAGAAGATTCGAAGACCTCCACTTCAGTCACCAGCGTGAAC	2340
2341	CAGGCGAGCACGTCACGCCTGGAGGGACTGCAGTCAGAAAACCACCGCCTTCGAATGAAG	2400
2401	ATCACAGAGCTGGACAAAGACTTGGAAGAAGTCACCATGCAGCTACAAGACACACCAGAG	2460
2461	AAGACCACATACATCAAACAGAATCACTACCAAGAGCTCAACGACATCCTCAGCTTGGGC	2520

FIGURE 3D

2521 AACTTCACAGAGAGCACAGATGGAGGAAAGGCCATTCTAAAAAATCACCTCGATCAAAAC 2580

2581 CCCCAGCTCCAGTGGAACACGACAGAGCCCTCAAGAACATGCAAAGACCCCATAGAAGAC 2640

2641 ATCAACTCCCCGGAGCACATCCAGCGCCGGCTGTCGCTCCAGCTCCCCATCCTTCACCAC 2700

2701 GCCTACCTCCCATCCATCGGAGGCGTGGATGCCAGCTGCGTCAGCCCCTGTGTCAGCCCT 2760

2761 ACCGCCAGCCCTCGCCACAGACACGTACCACCCTCCTTCCGAGTCATGGTCTCGGGCCTG 2820

2821 TAG 2823

FIGURE 4A

1	M	A	S	P	P	S	S	G	Q	P	R	P	P	P	P	P	P	P	A	20
21	R	L	L	L	P	L	L	L	S	L	L	L	W	L	A	P	G	A	W	G	40
41	W	T	R	G	A	P	R	P	P	P	S	S	P	P	L	S	I	M	G	L	60
61	M	P	L	T	K	E	V	A	K	G	S	I	G	R	G	V	L	P	A	V	80
81	E	L	A	I	E	Q	I	R	N	E	S	L	L	R	P	Y	F	L	D	L	100
101	R	L	Y	D	T	E	C	D	N	A	K	G	L	K	A	F	Y	D	A	I	120
121	K	Y	G	P	N	H	L	M	V	F	G	G	V	C	P	S	V	T	S	I	140
141	I	A	E	S	L	Q	G	W	N	L	V	Q	L	S	F	A	A	T	T	P	160
161	V	L	A	D	K	K	K	Y	P	Y	F	F	R	T	V	P	S	D	N	A	180
181	V	N	P	A	I	L	K	L	L	K	H	F	R	W	R	R	V	G	T	L	200
201	T	Q	D	V	Q	R	F	S	E	V	R	N	D	L	T	G	V	L	Y	G	220
221	E	D	I	E	I	S	D	T	E	S	F	S	N	D	P	C	T	S	V	K	240
241	K	L	K	G	N	D	V	R	I	I	L	G	Q	F	D	Q	N	M	A	A	260

FIGURE 4B

261	K	V	F	C	C	A	F	E	E	S	M	F	G	S	K	Y	Q	W	I	I	280
281	P	G	W	Y	E	P	A	W	W	E	Q	V	H	V	E	A	N	S	S	R	300
301	C	L	R	R	S	L	L	A	A	M	E	G	Y	I	G	V	D	F	E	P	320
321	L	S	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	E	R	E	340
341	Y	N	S	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	Y	D	G	360
361	I	W	V	I	A	K	T	L	Q	R	A	M	E	T	L	H	A	S	S	R	380
381	H	Q	R	I	Q	D	F	N	Y	T	D	H	T	L	G	K	I	I	L	N	400
401	A	M	N	E	T	N	F	F	G	V	T	G	Q	V	V	F	R	N	G	E	420
421	R	M	G	T	I	K	F	T	Q	F	Q	D	S	R	E	V	K	V	G	E	440
441	Y	N	A	V	A	D	T	L	E	I	I	N	D	T	I	R	F	Q	G	S	460
461	E	P	P	K	D	K	T	I	I	L	E	Q	L	R	K	I	S	L	P	L	480
481	Y	S	I	L	S	A	L	T	I	L	G	M	I	M	A	S	A	F	L	F	500
501	F	N	I	K	N	R	N	Q	K	L	I	K	M	S	S	P	Y	M	N	N	520
521	L	I	I	L	G	G	M	L	S	Y	A	S	I	F	L	F	G	L	D	G	540

FIGURE 4C

541	S	F	V	S	E	K	T	F	E	T	L	C	T	V	R	T	W	I	L	T	560
561	V	G	Y	T	T	A	F	G	A	M	F	A	K	T	W	R	V	H	A	I	580
581	F	K	N	V	K	M	K	K	K	I	I	K	D	Q	K	L	L	V	I	V	600
601	G	G	M	L	L	I	D	L	C	I	L	I	C	W	Q	A	V	D	P	L	620
621	R	R	T	V	E	R	Y	S	M	E	P	D	P	A	G	R	D	I	S	I	640
641	R	P	L	L	E	H	C	E	N	T	H	M	T	I	W	L	G	I	V	Y	660
661	A	Y	K	G	L	L	M	L	F	G	C	F	L	A	W	E	T	R	N	V	680
681	S	I	P	A	L	N	D	S	K	Y	I	G	M	S	V	Y	N	V	G	I	700
701	M	C	I	I	G	A	A	V	S	F	L	T	R	D	Q	P	N	V	Q	F	720
721	C	I	V	A	L	V	I	I	F	C	S	T	I	T	L	C	L	V	F	V	740
741	P	K	L	I	T	L	R	T	N	P	D	A	A	T	Q	N	R	R	F	Q	760
761	F	T	Q	N	Q	K	K	E	D	S	K	T	S	T	S	V	T	S	V	N	780
781	Q	A	S	T	S	R	L	E	G	L	Q	S	E	N	H	R	L	R	M	K	800
801	I	T	E	L	D	K	D	L	E	E	V	T	M	Q	L	Q	D	T	P	E	820

FIGURE 4D

821	K	T	T	Y	I	K	Q	N	H	Y	Q	E	L	N	D	I	L	S	L	G	840
841	N	F	T	E	S	T	D	G	G	K	A	I	L	K	N	H	L	D	Q	N	860
861	P	Q	L	Q	W	N	T	T	E	P	S	R	T	C	K	D	P	I	E	D	880
881	I	N	S	P	E	H	I	Q	R	R	L	S	L	Q	L	P	I	L	H	H	900
901	A	Y	L	P	S	I	G	G	V	D	A	S	C	V	S	P	C	V	S	P	920
921	T	A	S	P	R	H	R	H	V	P	P	S	F	R	V	M	V	S	G	L	940

FIGURE 5A

1	M	P	S	C	P	A	R	S	A	T	G	P	L	S	I	M	G	L	M	P	20
21	L	T	K	E	V	A	K	G	S	I	G	R	G	V	L	P	A	V	E	L	40
41	A	I	E	Q	I	R	N	E	S	L	L	R	P	Y	F	L	D	L	R	L	60
61	Y	D	T	E	C	D	N	A	K	G	L	K	A	F	Y	D	A	I	K	Y	80
81	G	P	N	H	L	M	V	F	G	G	V	C	P	S	V	T	S	I	I	A	100
101	E	S	L	Q	G	W	N	L	V	Q	L	S	F	A	A	T	T	P	V	L	120
121	A	D	K	K	K	Y	P	Y	F	F	R	T	V	P	S	D	N	A	V	N	140
141	P	A	I	L	K	L	L	K	H	Y	Q	W	K	R	V	G	T	L	T	Q	160
161	D	V	Q	R	F	S	E	V	R	N	D	L	T	G	V	L	Y	G	E	D	180
181	I	E	I	S	D	T	E	S	F	S	N	D	P	C	T	S	V	K	K	L	200
201	K	G	N	D	V	R	I	I	L	G	Q	F	D	Q	N	M	A	A	K	V	220
221	F	C	C	A	Y	E	E	N	M	Y	G	S	K	Y	Q	W	I	I	P	G	240
241	W	Y	E	P	S	W	W	E	Q	V	H	T	E	A	N	S	S	R	C	L	260
261	R	K	N	L	L	A	A	M	E	G	Y	I	G	V	D	F	E	P	L	S	280
281	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	E	R	E	Y	N	300
301	N	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	Y	D	G	I	W	320

FIGURE 5B

```
321  V I A K T L Q R A M E T L H A S S R H Q  340
341  R I Q D F N Y T D H T L G R I I L N A M  360
361  N E T N F F G V T G Q V V F R N G E R M  380
381  G T I K F T Q F Q D S R E V K V G E Y N  400
401  A V A D T L E I I N D T I R F Q G S E P  420

                                    ⌜──────
421  P K D K T I I L E Q L R K I S L P L Y S  440
     ──────────────── I ────────────────────
441  I L S A L T I L G M I M A S A F L F F N  460
     ⌐                   ⌜──────────────────
461  I K N R N Q K L I K M S S P Y M N N L I  480
     ──────────────── II ───────────────────
481  I L G G M L S Y A S I F L F G L D G S F  500
```

FIGURE 5C

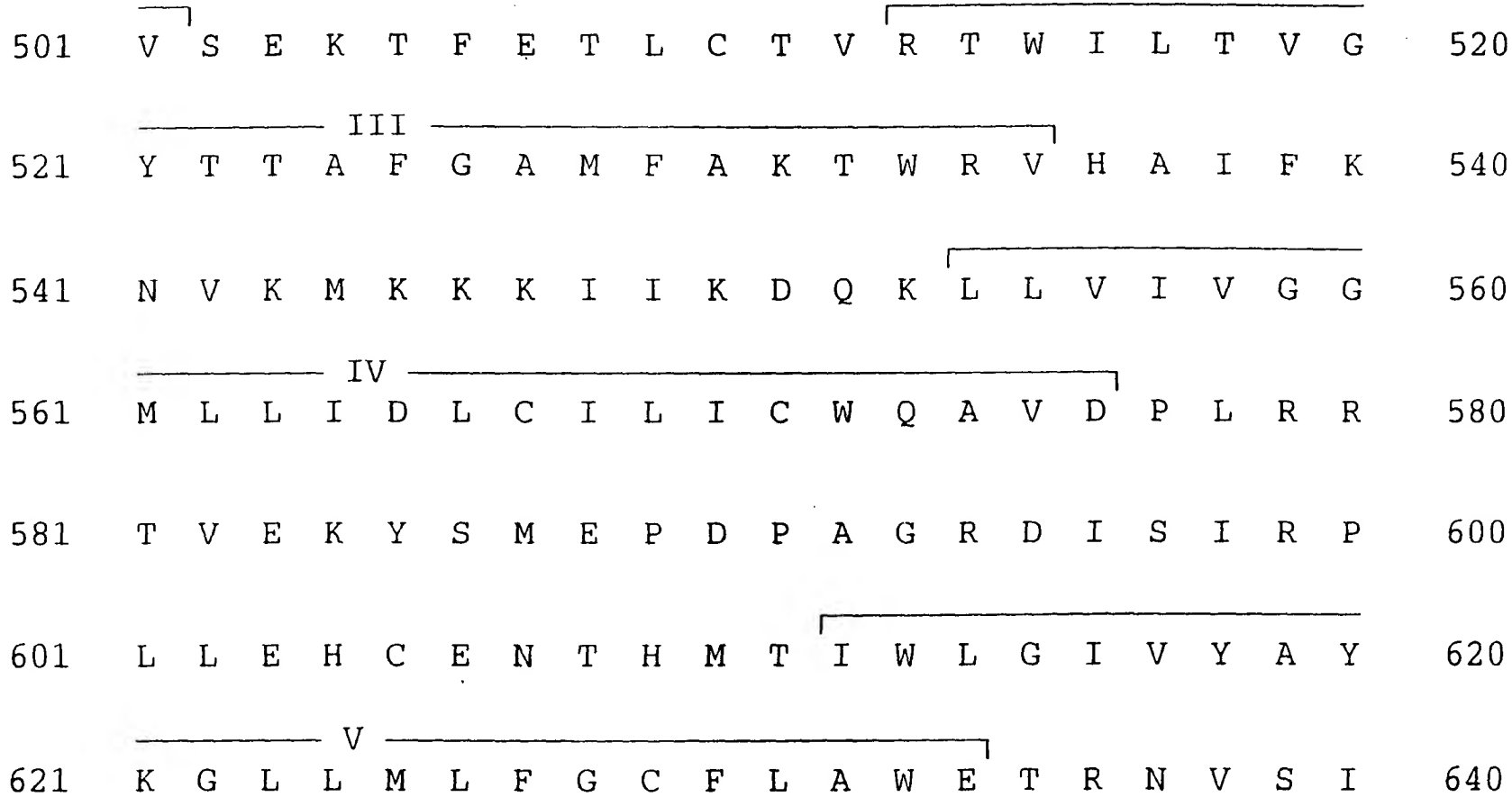

III
IV
V
501 V S E K T F E T L C T V R T W I L T V G 520
521 Y T T A F G A M F A K T W R V H A I F K 540
541 N V K M K K K I I K D Q K L L V I V G G 560
561 M L L I D L C I L I C W Q A V D P L R R 580
581 T V E K Y S M E P D P A G R D I S I R P 600
601 L L E H C E N T H M T I W L G I V Y A Y 620
621 K G L L M L F G C F L A W E T R N V S I 640

FIGURE 5D

641	P	A	L	N	D	S	K	Y	I	G	M	S	V	Y	N	V	G	I	M	C	660
				VI																	
661	I	I	G	A	A	V	S	F	L	T	R	D	Q	P	N	V	Q	F	C	I	680
				VII																	
681	V	A	L	V	I	I	F	C	S	T	I	T	L	C	L	V	F	V	P	K	700
701	L	I	T	L	R	T	N	P	D	A	A	T	Q	N	R	R	F	Q	F	T	720
721	Q	N	Q	K	K	E	D	S	K	T	S	T	S	V	T	S	V	N	Q	A	740
741	S	T	S	R	L	E	G	L	Q	S	E	N	H	R	L	R	M	K	I	T	760
761	E	L	D	K	D	L	E	E	V	T	M	Q	L	Q	D	T	P	E	K	T	780
781	T	Y	I	K	Q	N	H	Y	Q	E	L	N	D	I	L	N	L	G	N	F	800
801	T	E	S	T	D	G	G	K	A	I	L	K	N	H	L	D	Q	N	P	Q	820
821	L	Q	W	N	T	T	E	P	S	R	T	C	K	D	P	I	E	D	I	N	840
841	S	P	E	H	I	Q	R	R	L	S	L	Q	L	P	I	L	H	H	A	Y	860
861	L	P	S	I	G	G	V	D	A	S	C	V	S	P	C	V	S	P	T	A	880
881	S	P	R	H	R	H	V	P	P	S	F	R	V	M	V	S	G	L			898

Figure 6A

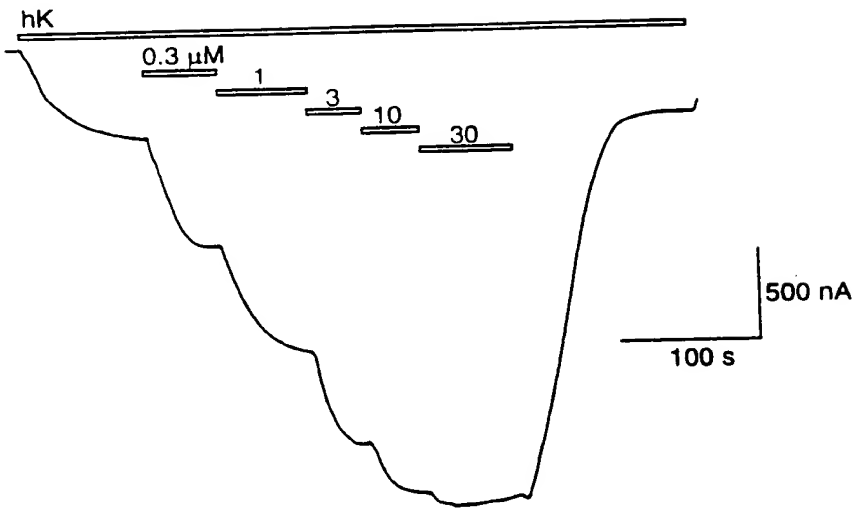


Figure 6B

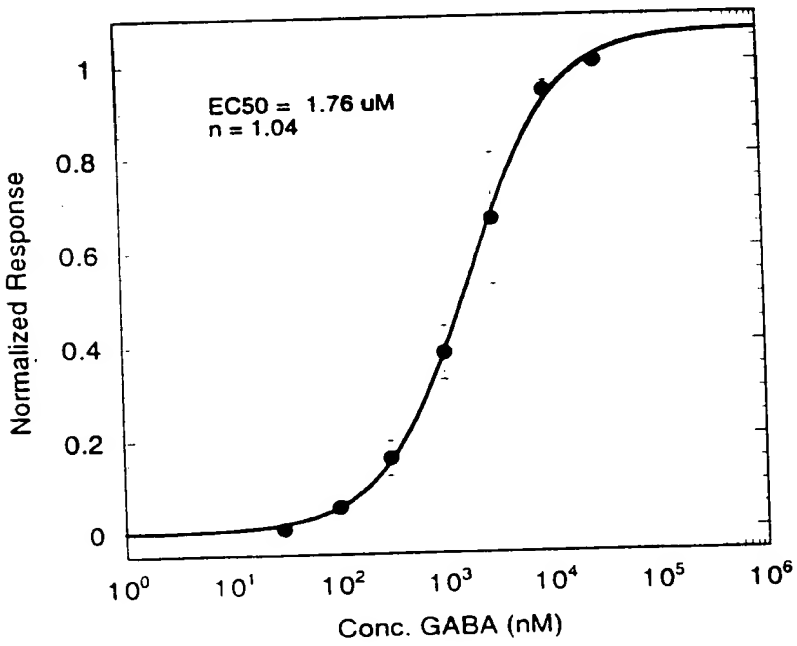


Figure 7

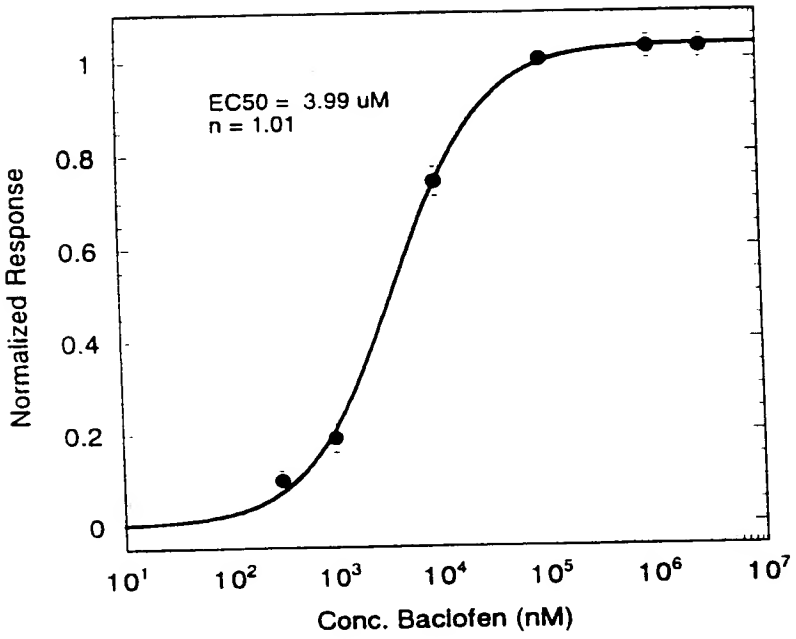

EC50 = 3.99 uM
n = 1.01
Normalized Response
1
0.8
0.6
0.4
0.2
0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
Conc. Baclofen (nM)

Figure 8

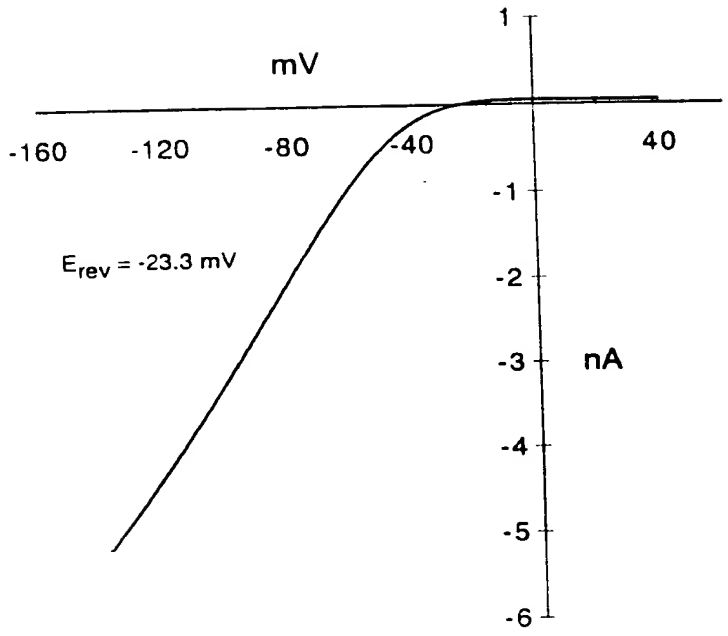


Figure 9A

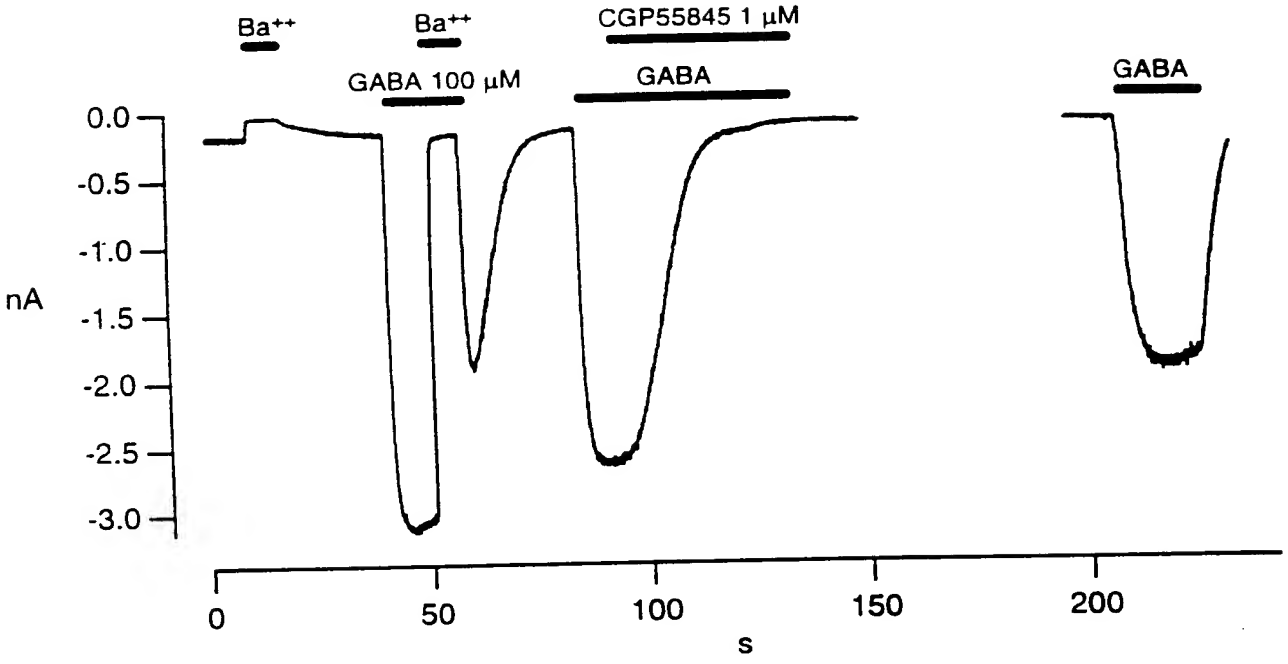
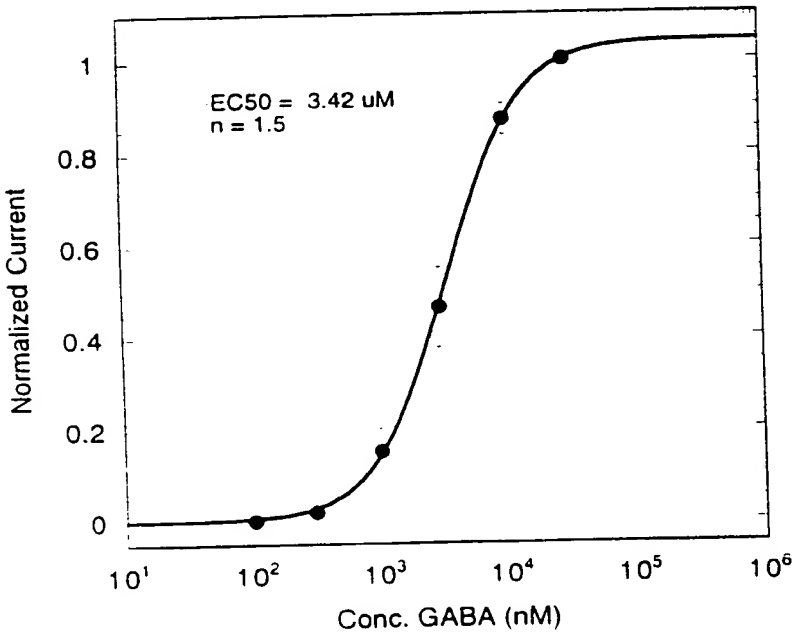


Figure 9B



Figur 10

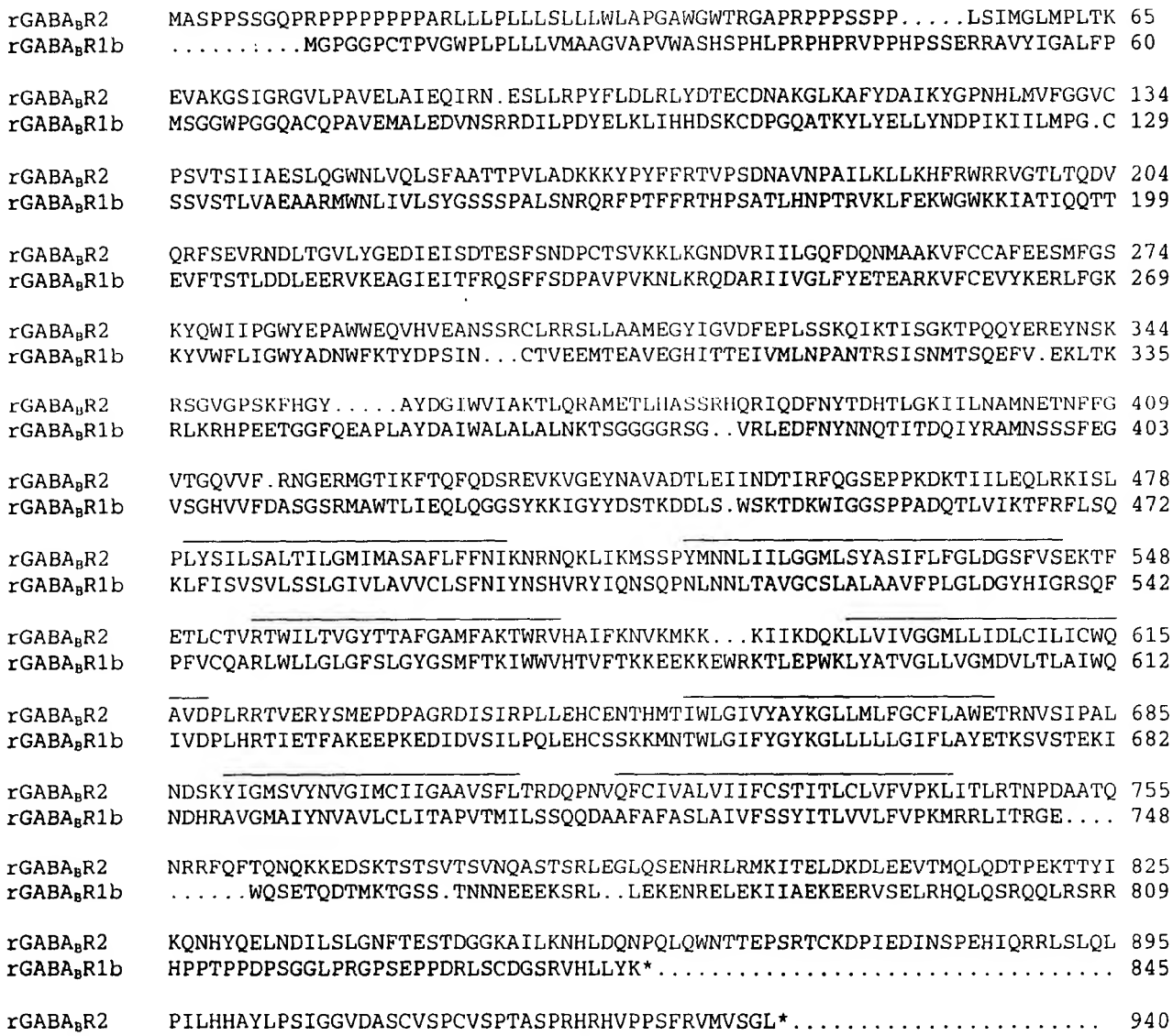

rGABA$_B$R2 MASPPSSGQPRPPPPPPPPARLLLPLLLSLLLWLAPGAWGWTRGAPRPPPSSPP.....LSIMGLMPLTK 65
rGABA$_B$R1b .....:...MGPGGPCTPVGWPLPLLLVMAAGVAPVWASHSPHLPRPHPRVPPHPSSERRAVYIGALFP 60
rGABA$_B$R2 EVAKGSIGRGVLPAVELAIEQIRN.ESLLRPYFLDLRLYDTECDNAKGLKAFYDAIKYGPNHLMVFGGVC 134
rGABA$_B$R1b MSGGWPGGQACQPAVEMALEDVNSRRDILPDYELKLIHHDSKCDPGQATKYLYELLYNDPIKIILMPG.C 129
rGABA$_B$R2 PSVTSIIAESLQGWNLVQLSFAATTPVLADKKKYPYFFRTVPSDNAVNPAILKLLKHFRWRRVGTLTQDV 204
rGABA$_B$R1b SSVSTLVAEAARMWNLIVLSYGSSSPALSNRQRFPTFFRTHPSATLHNPTRVKLFEKWGWKKIATIQQTT 199
rGABA$_B$R2 QRFSEVRNDLTGVLYGEDIEISDTESFSNDPCTSVKKLKGNDVRIILGQFDQNMAAKVFCCAFEESMFGS 274
rGABA$_B$R1b EVFTSTLDDLEERVKEAGIEITFRQSFFSDPAVPVKNLKRQDARIIVGLFYETEARKVFCEVYKERLFGK 269
rGABA$_B$R2 KYQWIIPGWYEPAWWEQVHVEANSSRCLRRSLLAAMEGYIGVDFEPLSSKQIKTISGKTPQQYEREYNSK 344
rGABA$_B$R1b KYVWFLIGWYADNWFKTYDPSIN...CTVEEMTEAVEGHITTEIVMLNPANTRSISNMTSQEFV.EKLTK 335
rGABA$_B$R2 RSGVGPSKFHGY.....AYDGIWVIAKTLQRAMETLHASSRHQRIQDFNYTDHTLGKIILNAMNETNFFG 409
rGABA$_B$R1b RLKRHPEETGGFQEAPLAYDAIWALALALNKTSGGGGRSG..VRLEDFNYNNQTITDQIYRAMNSSSFEG 403
rGABA$_B$R2 VTGQVVF.RNGERMGTIKFTQFQDSREVKVGEYNAVADTLEIINDTIRFQGSEPPKDKTIILEQLRKISL 478
rGABA$_B$R1b VSGHVVFDASGSRMAWTLIEQLQGGSYKKIGYYDSTKDDLS.WSKTDKWIGGSPPADQTLVIKTFRFLSQ 472
rGABA$_B$R2 PLYSILSALTILGMIMASAFLFFNIKNRNQKLIKMSSPYMNNLIILGGMLSYASIFLFGLDGSFVSEKTF 548
rGABA$_B$R1b KLFISVSVLSSLGIVLAVVCLSFNIYNSHVRYIQNSQPNLNNLTAVGCSLALAAVFPLGLDGYHIGRSQF 542
rGABA$_B$R2 ETLCTVRTWILTVGYTTAFGAMFAKTWRVHAIFKNVKMKK...KIIKDQKLLVIVGGMLLIDLCILICWQ 615
rGABA$_B$R1b PFVCQARLWLLGLGFSLGYGSMFTKIWWVHTVFTKKEEKKEWRKTLEPWKLYATVGLLVGMDVLTLAIWQ 612
rGABA$_B$R2 AVDPLRRTVERYSMEPDPAGRDISIRPLLEHCENTHMTIWLGIVYAYKGLLMLFGCFLAWETRNVSIPAL 685
rGABA$_B$R1b IVDPLHRTIETFAKEEPKEDIDVSILPQLEHCSSKKMNTWLGIFYGYKGLLLLLGIFLAYETKSVSTEKI 682
rGABA$_B$R2 NDSKYIGMSVYNVGIMCIIGAAVSFLTRDQPNVQFCIVALVIIFCSTITLCLVFVPKLITLRTNPDAATQ 755
rGABA$_B$R1b NDHRAVGMAIYNVAVLCLITAPVTMILSSQQDAAFAFASLAIVFSSYITLVVLFVPKMRRLITRGE.... 748
rGABA$_B$R2 NRRFQFTQNQKKEDSKTSTSVTSVNQASTSRLEGLQSENHRLRMKITELDKDLEEVTMQLQDTPEKTTYI 825
rGABA$_B$R1b .....WQSETQDTMKTGSS.TNNNEEEKSRL..LEKENRELEKIIAEKEERVSELRHQLQSRQQLRSRR 809
rGABA$_B$R2 KQNHYQELNDILSLGNFTESTDGGKAILKNHLDQNPQLQWNTTEPSRTCKDPIEDINSPEHIQRRLSLQL 895
rGABA$_B$R1b HPPTPPDPSGGLPRGPSEPPDRLSCDGSRVHLLYK*..... 845
rGABA$_B$R2 PILHHAYLPSIGGVDASCVSPCVSPTASPRHRHVPPSFRVMVSGL*..... 940

Figure 11A

Figure 11B

Figure 11C

Figure 11D

Figure 12A

Figure 12B

Figure 13A

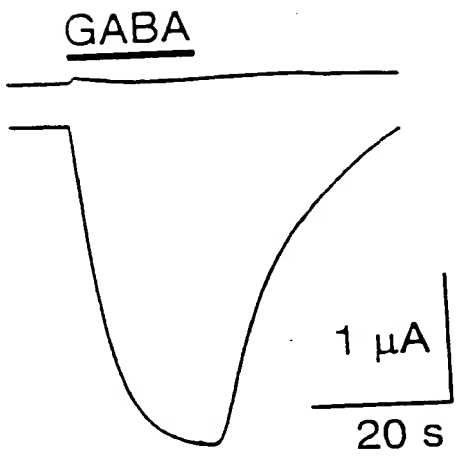


Figure 13B

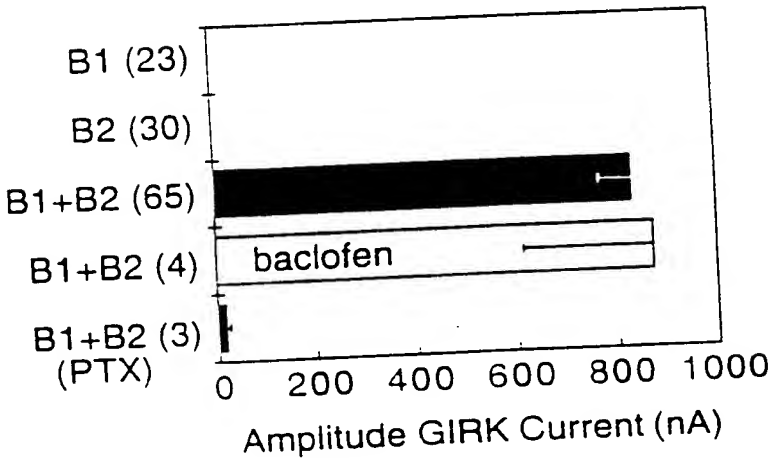


Figure 14A

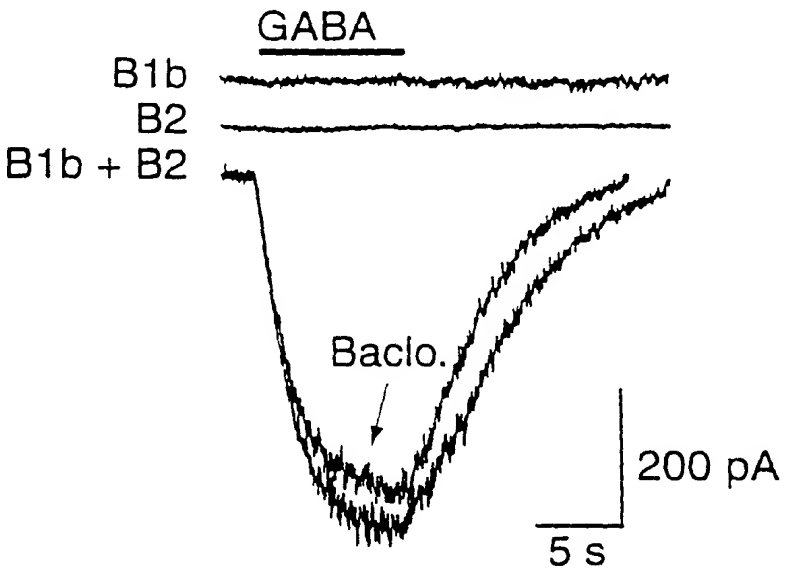


Figure 14B

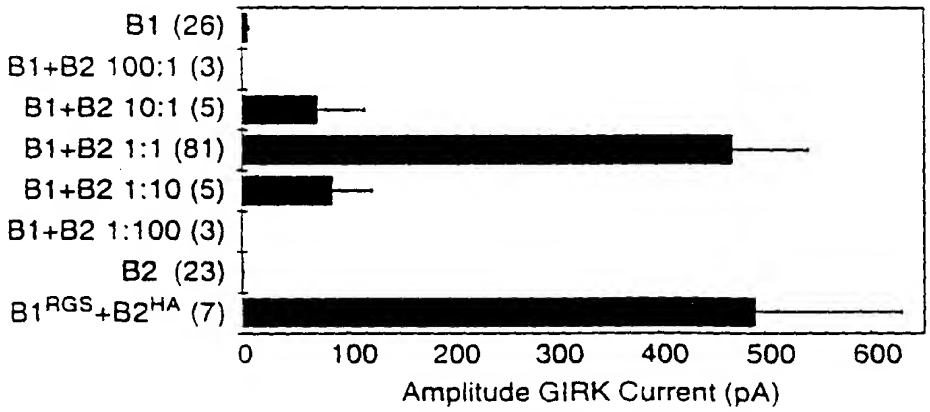


Figure 15A

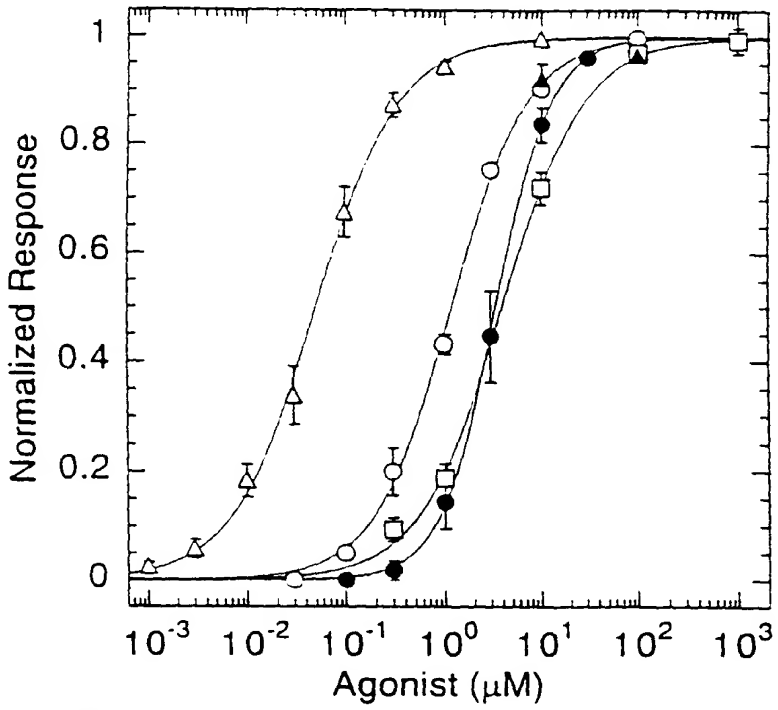


Figure 15B

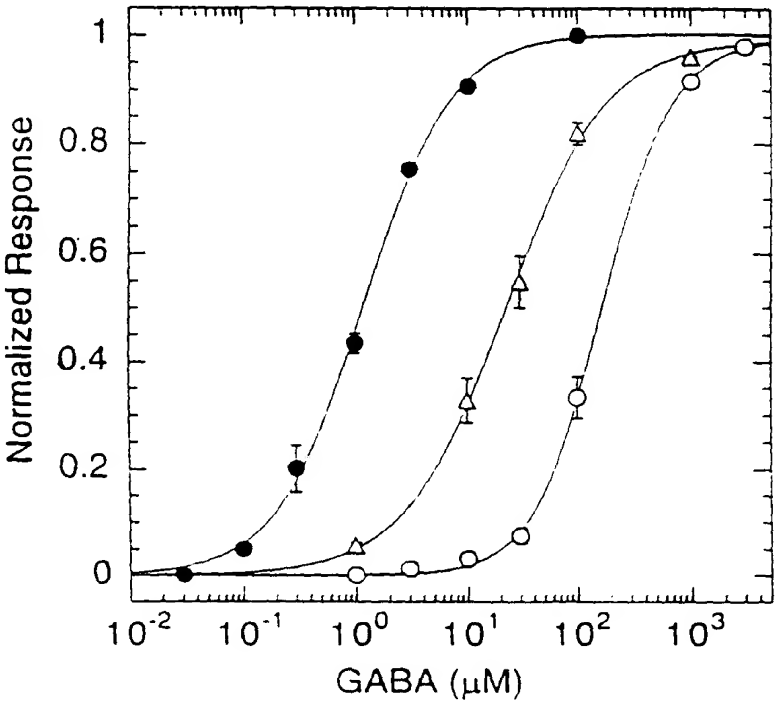


Figure 16

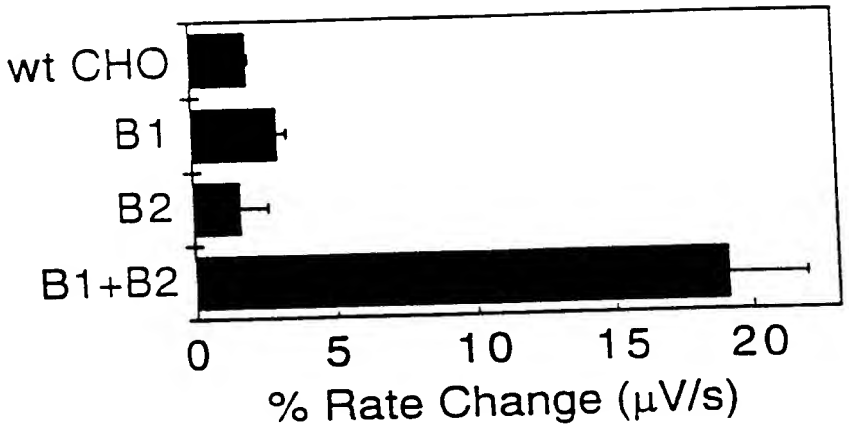


Figure 17A

Figure 17B

Figure 17C

Figure 17D

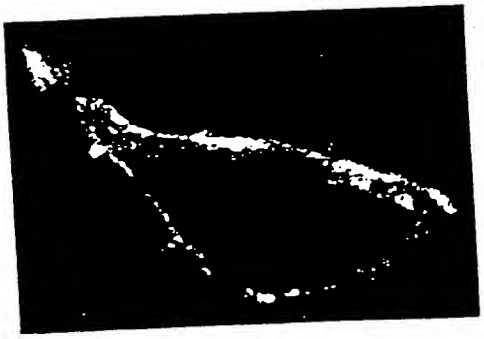


Figure 18A

Figure 18B

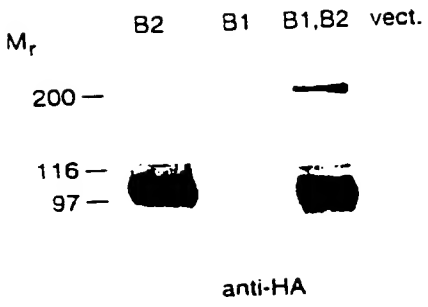
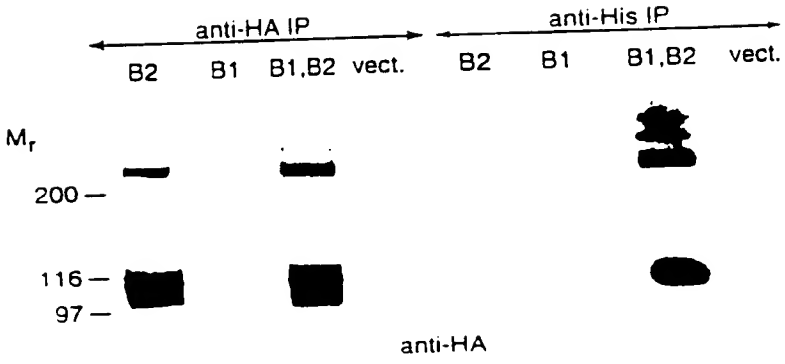


Figure 18C



Silver grain density:

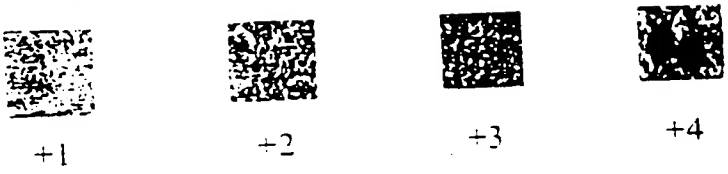


FIGURE 19A

FIGURE 19B

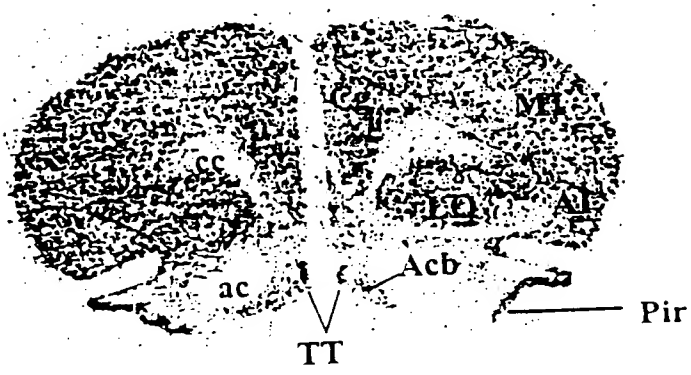


FIGURE 19C

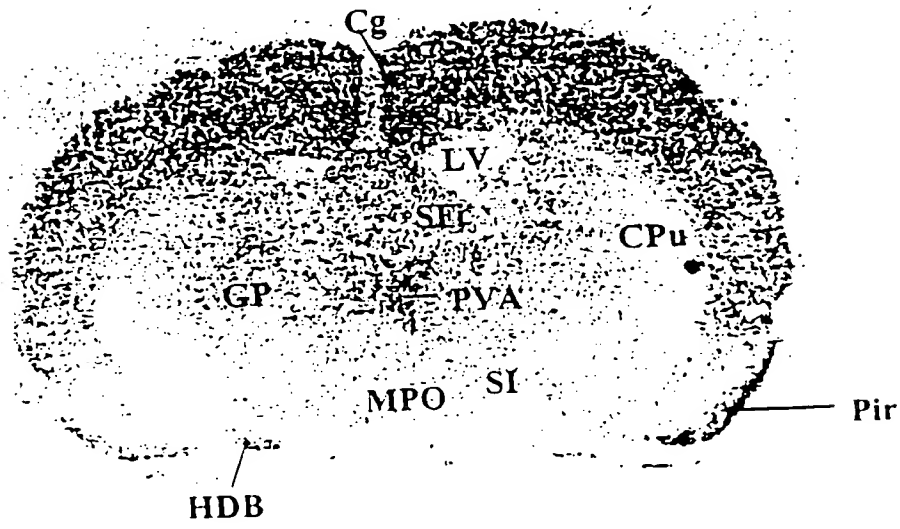


FIGURE 19D

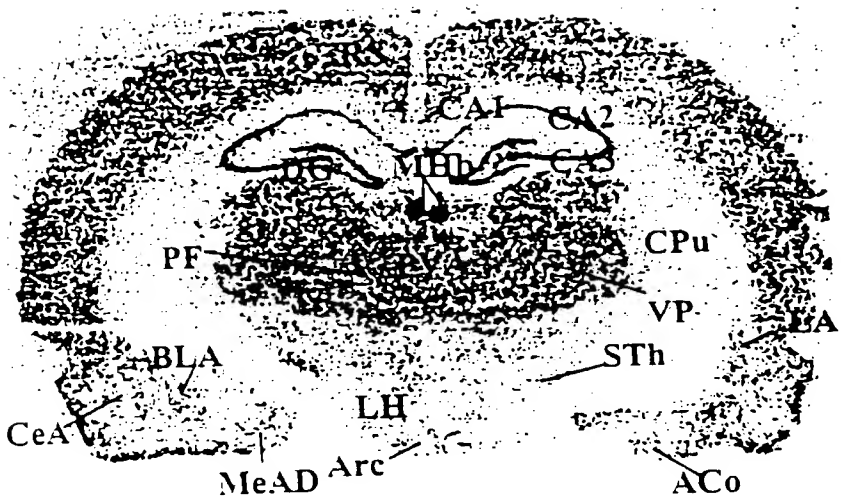


FIGURE 19E

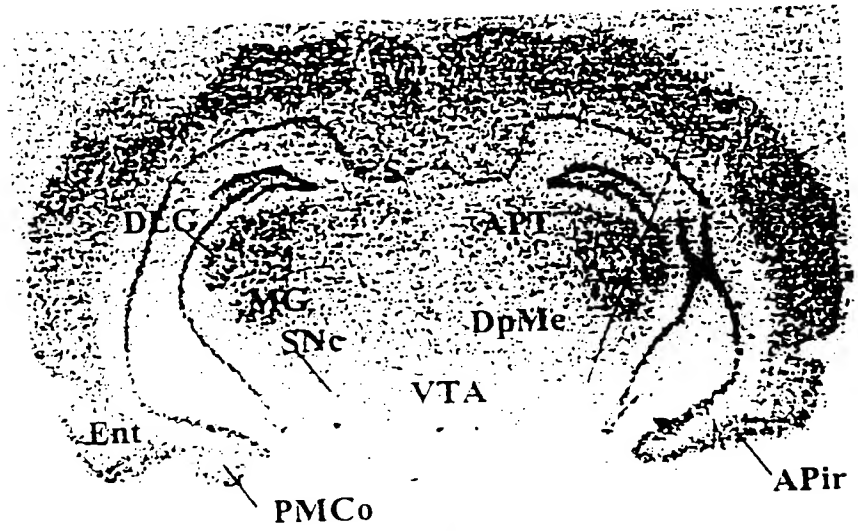


FIGURE 19F

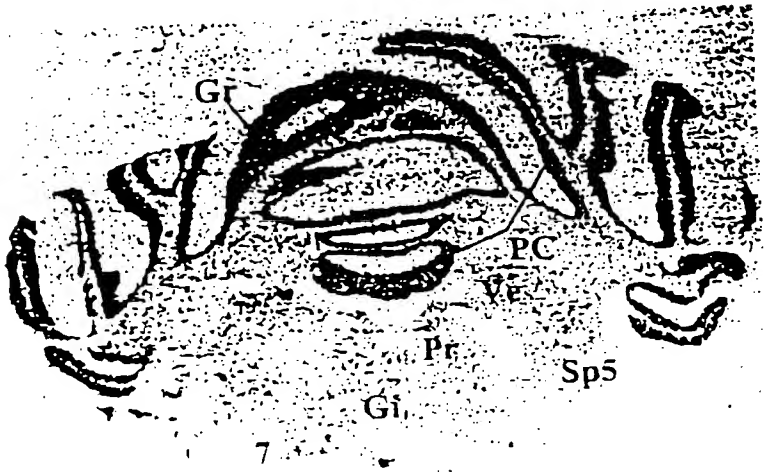


FIGURE 19G

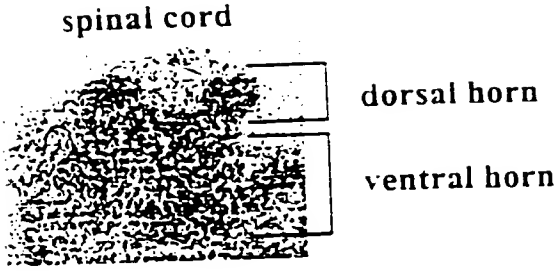


FIGURE 19H

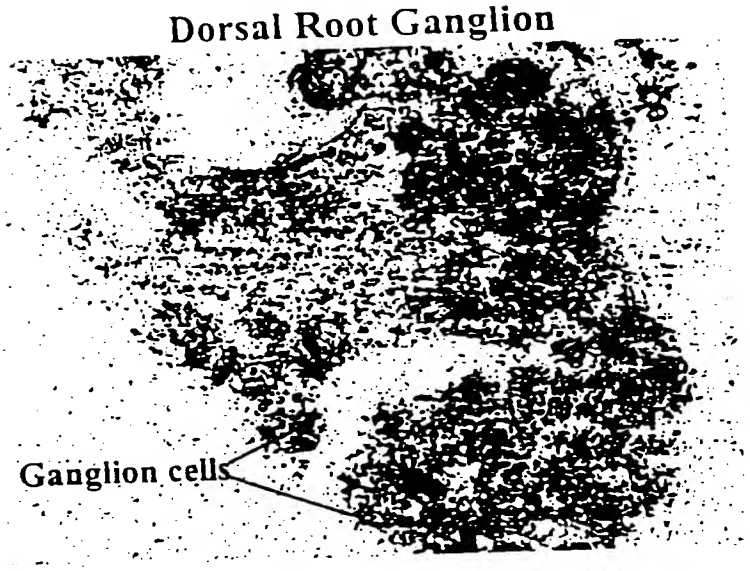


FIGURE 19I

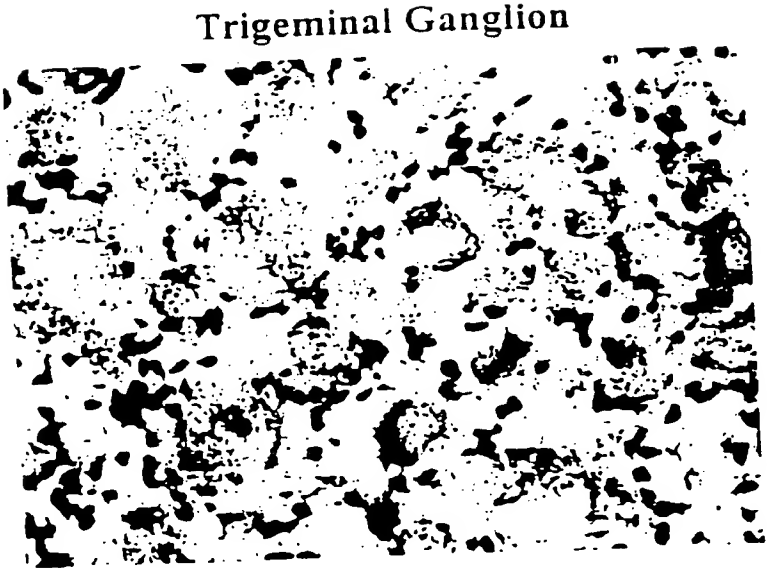


Figure 20A

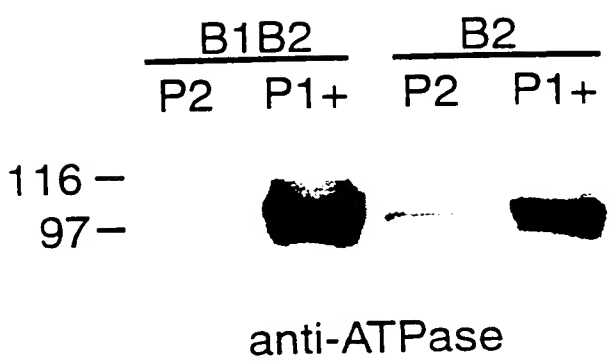


Figure 20B

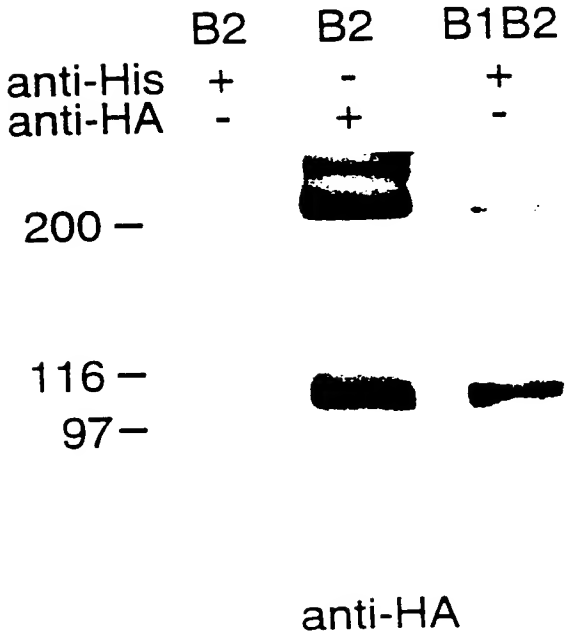


Figure 20C

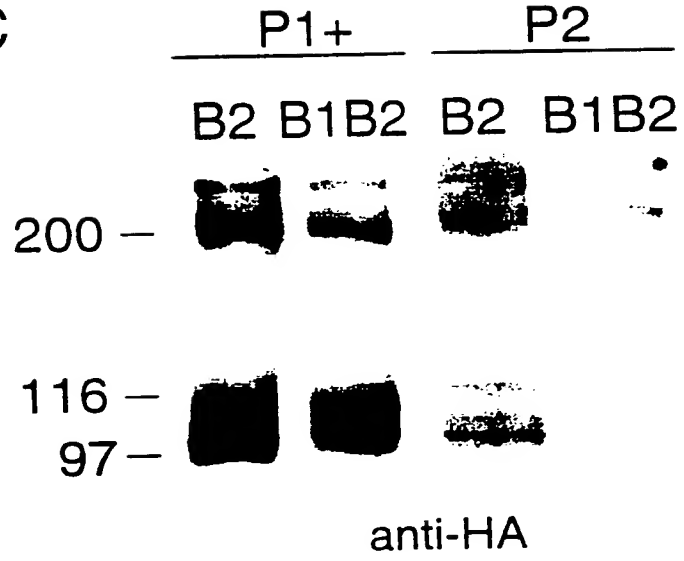


Figure 21A

Figure 21B

Figure 21C

Figure 21D

Figure 21E

Figure 21F

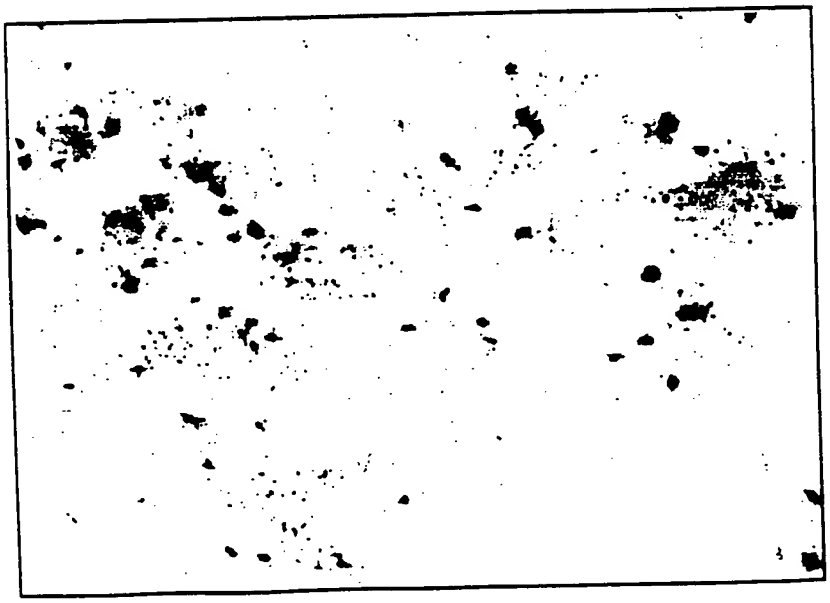


FIGURE 22A

```
   1  ATGGCTTCCCCGCGGAGCTCCGGGCAGCCCGGGCCGCCGCCGCCGCCACCGCCGCCC   60
  61  GCGCGCCTGCTACTGCTACTGCTGCTGCCGCTGCTGCTGCCTCTGGCGCCCGGGGCCTGG  120
 121  GGCTGGGCGCGGGGCGCCCCCCGGCCGCCGCCCAGCAGCCCGCCGCTCTCCATCATGGGC  180
 181  CTCATGCCGCTCACCAAGGAGGTGGCCAAGGGCAGCATCGGGCGCGGTGTGCTCCCCGCC  240
 241  GTGGAACTGGCCATCGAGCAGATCCGCAACGAGTCACTCCTGCGCCCCTACTTCCTCGAC  300
 301  CTGCGGCTCTATGACACGGAGTGCGACAACGCAAAAGGGTTGAAAGCCTTCTACGATGCG  360
 361  ATAAAATACGGGCCGAACCACTTGATGGTGTTTGGAGGCGTCTGTCCATCCGTCACATCC  420
 421  ATCATTGCAGAGTCCCTCCAAGGCTGGAATCTGGTGCAGCTTTCTTTTGCTGCAACCACG  480
 481  CCTGTTCTAGCCGATAAGAAAAAATACCCTTATTTCTTTCGGACCGTCCCATCAGACAAT  540
 541  GCGGTGAATCCAGCCATTCTGAAGTTGCTCAAGCACTACCAGTGGAAGCGCGTGGGCACG  600
 601  CTGACGCAAGACGTTCAGAGGTTCTCTGAGGTGCGGAATGACCTGACTGGAGTTCTGTAT  660
 661  GGCGAGGACATTGAGATTTCAGACACCGAGAGCTTCTCCAACGATCCCTGTACCAGTGTC  720
 721  AAAAAGCTGAAGGGGAATGATGTGCGGATCATCCTTGGCCAGTTTGACCAGAATATGGCA  780
 781  GCAAAAGTGTTCTGTTGTGCATACGAGGAGAACATGTATGGTAGTAAATATCAGTGGATC  840
```

FIGURE 22B

```
841  ATTCCGGGCTGGTACGAGCCTTCTTGGTGGGAGCAGGTGCACACGGAAGCCAACTCATCC  900
901  CGCTGCCTCCGGAAGAATCTGCTTGCTGCCATGGAGGGCTACATTGGCGTGGATTTCGAG  960
961  CCCCTGAGCTCCAAGCAGATCAAGACCATCTCAGGAAAGACTCCACAGCAGTATGAGAGA  1020
1021 GAGTACAACAACAAGCGGTCAGGCGTGGGGCCCAGCAAGTTCCACGGGTACGCCTACGAT  1080
1081 GGCATCTGGGTCATCGCCAAGACACTGCAGAGGGCCATGGAGACACTGCATGCCAGCAGC  1140
1141 CGGCACCAGCGGATCCAGGACTTCAACTACACGGACCACACGCTGGGCAGGATCATCCTC  1200
1201 AATGCCATGAACGAGACCAACTTCTTCGGGGTCACGGGTCAAGTTGTATTCCGGAATGGG  1260
1261 GAGAGAATGGGGACCATTAAATTTACTCAATTTCAAGACAGCAGGGAGGTGAAGGTGGGA  1320
1321 GAGTACAACGCTGTGGCCGACACACTGGAGATCATCAATGACACCATCAGGTTCCAAGGA  1380
1381 TCCGAACCACCAAAAGACAAGACCATCATCCTGGAGCAGCTGCGGAAGATCTCCCTACCT  1440
1441 CTCTACAGCATCCTCTCTGCCCTCACCATCCTCGGGATGATCATGGCCAGTGCTTTTCTC  1500
1501 TTCTTCAACATCAAGAACCGGAATCAGAAGCTCATAAAGATGTCGAGTCCATACATGAAC  1560
1561 AACCTTATCATCCTTGGAGGGATGCTTTCCTATGCTTCCATATTTCTCTTTGGCCTTGAT  1620
1621 GGATCCTTTGTCTCTGAAAAGACCTTTGAAACACTTTGCACCGTCAGGACCTGGATTCTC  1680
```

FIGURE 22C

1681	ACCGTGGGCTACACGACCGCTTTTGGGGCCATGTTTGCAAAGACCTGGAGAGTCCACGCC	1740
1741	ATCTTCAAAAATGTGAAAATGAAGAAGAAGATCATCAAGGACCAGAAACTGCTTGTGATC	1800
1801	GTGGGGGGCATGCTGCTGATCGACCTGTGTATCCTGATCTGCTGGCAGGCTGTGGACCCC	1860
1861	CTGCGAAGGACAGTGGAGAAGTACAGCATGGAGCCGGACCCAGCAGGACGGGATATCTCC	1920
1921	ATCCGCCCTCTCCTGGAGCACTGTGAGAACACCCATATGACCATCTGGCTTGGCATCGTC	1980
1981	TATGCCTACAAGGGACTTCTCATGTTGTTCGGTTGTTTCTTAGCTTGGGAGACCCGCAAC	2040
2041	GTCAGCATCCCCGCACTCAACGACAGCAAGTACATCGGGATGAGTGTCTACAACGTGGGG	2100
2101	ATCATGTGCATCATCGGGGCCGCTGTCTCCTTCCTGACCCGGGACCAGCCCAATGTGCAG	2160
2161	TTCTGCATCGTGGCTCTGGTCATCATCTTCTGCAGCACCATCACCCTCTGCCTGGTATTC	2220
2221	GTGCCGAAGCTCATCACCCTGAGAACAAACCCAGATGCAGCAACGCAGAACAGGCGATTC	2280
2281	CAGTTCACTCAGAATCAGAAGAAAGAAGATTCTAAAACGTCCACCTCGGTCACCAGTGTG	2340
2341	AACCAAGCCAGCACATCCCGCCTGGAGGGCCTACAGTCAGAAAACCATCGCCTGCGAATG	2400
2401	AAGATCACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACACACCA	2460
2461	GAAAAGACCACCTACATTAAACAGAACCACTACCAAGAGCTCAATGACATCCTCAACCTG	2520

FIGURE 22D

2521 GGAAACTTCACTGAGAGCACAGATGGAGGAAAGGCCATTTTAAAAAATCACCTCGATCAA 2580

2581 AATCCCCAGCTACAGTGGAACACAACAGAGCCCTCTCGAACATGCAAAGATCCTATAGAA 2640

2641 GATATAAACTCTCCAGAACACATCCAGCGTCGGCTGTCCCTCCAGCTCCCCATCCTCCAC 2700

2701 CACGCCTACCTCCCATCCATCGGAGGCGTGGACGCCAGCTGTGTCAGCCCCTGCGTCAGC 2760

2761 CCCACCGCCAGCCCCCGCCACAGACATGTGCCACCCTCCTTCCGAGTCATGGTCTCGGGC 2820

2821 CTGTAA 2826

FIGURE 23A

1	M	A	S	P	R	S	S	G	Q	P	G	P	P	P	P	P	P	P	P	20
21	A	R	L	L	L	L	L	L	L	P	L	L	L	P	L	A	P	G	A	W	40
41	G	W	A	R	G	A	P	R	P	P	P	S	S	P	P	L	S	I	M	G	60
61	L	M	P	L	T	K	E	V	A	K	G	S	I	G	R	G	V	L	P	A	80
81	V	E	L	A	I	E	Q	I	R	N	E	S	L	L	R	P	Y	F	L	D	100
101	L	R	L	Y	D	T	E	C	D	N	A	K	G	L	K	A	F	Y	D	A	120
121	I	K	Y	G	P	N	H	L	M	V	F	G	G	V	C	P	S	V	T	S	140
141	I	I	A	E	S	L	Q	G	W	N	L	V	Q	L	S	F	A	A	T	T	160
161	P	V	L	A	D	K	K	K	Y	P	Y	F	F	R	T	V	P	S	D	N	180
181	A	V	N	P	A	I	L	K	L	L	K	H	Y	Q	W	K	R	V	G	T	200
201	L	T	Q	D	V	Q	R	F	S	E	V	R	N	D	L	T	G	V	L	Y	220
221	G	E	D	I	E	I	S	D	T	E	S	F	S	N	D	P	C	T	S	V	240
241	K	K	L	K	G	N	D	V	R	I	I	L	G	Q	F	D	Q	N	M	A	260
261	A	K	V	F	C	C	A	Y	E	E	N	M	Y	G	S	K	Y	Q	W	I	280

FIGURE 23B

281	I	P	G	W	Y	E	P	S	W	W	E	Q	V	H	T	E	A	N	S	S	300
301	R	C	L	R	K	N	L	L	A	A	M	E	G	Y	I	G	V	D	F	E	320
321	P	L	S	S	K	Q	I	K	T	I	S	G	K	T	P	Q	Q	Y	E	R	340
341	E	Y	N	N	K	R	S	G	V	G	P	S	K	F	H	G	Y	A	Y	D	360
361	G	I	W	V	I	A	K	T	L	Q	R	A	M	E	T	L	H	A	S	S	380
381	R	H	Q	R	I	Q	D	F	N	Y	T	D	H	T	L	G	R	I	I	L	400
401	N	A	M	N	E	T	N	F	F	G	V	T	G	Q	V	V	F	R	N	G	420
421	E	R	M	G	T	I	K	F	T	Q	F	Q	D	S	R	E	V	K	V	G	440
441	E	Y	N	A	V	A	D	T	L	E	I	I	N	D	T	I	R	F	Q	G	460
461	S	E	P	P	K	D	K	T	I	I	L	E	Q	L	R	K	I	S	L	P	480
481	L	Y	S	I	L	S	A	L	T	I	L	G	M	I	M	A	S	A	F	L	500
501	F	F	N	I	K	N	R	N	Q	K	L	I	K	M	S	S	P	Y	M	N	520
521	N	L	I	I	L	G	G	M	L	S	Y	A	S	I	F	L	F	G	L	D	540
541	G	S	F	V	S	E	K	T	F	E	T	L	C	T	V	R	T	W	I	L	560

FIGURE 23C

561	T	V	G	Y	T	T	A	F	G	A	M	F	A	K	T	W	R	V	H	A	580
581	I	F	K	N	V	K	M	K	K	K	I	I	K	D	Q	K	L	L	V	I	600
601	V	G	G	M	L	L	I	D	L	C	I	L	I	C	W	Q	A	V	D	P	620
621	L	R	R	T	V	E	K	Y	S	M	E	P	D	P	A	G	R	D	I	S	640
641	I	R	P	L	L	E	H	C	E	N	T	H	M	T	I	W	L	G	I	V	660
661	Y	A	Y	K	G	L	L	M	L	F	G	C	F	L	A	W	E	T	R	N	680
681	V	S	I	P	A	L	N	D	S	K	Y	I	G	M	S	V	Y	N	V	G	700
701	I	M	C	I	I	G	A	A	V	S	F	L	T	R	D	Q	P	N	V	Q	720
721	F	C	I	V	A	L	V	I	I	F	C	S	T	I	T	L	C	L	V	F	740
741	V	P	K	L	I	T	L	R	T	N	P	D	A	A	T	Q	N	R	R	F	760
761	Q	F	T	Q	N	Q	K	K	E	D	S	K	T	S	T	S	V	T	S	V	780
781	N	Q	A	S	T	S	R	L	E	G	L	Q	S	E	N	H	R	L	R	M	800
801	K	I	T	E	L	D	K	D	L	E	E	V	T	M	Q	L	Q	D	T	P	820
821	E	K	T	T	Y	I	K	Q	N	H	Y	Q	E	L	N	D	I	L	N	L	840

FIGURE 23D

841	G	N	F	T	E	S	T	D	G	G	K	A	I	L	K	N	H	L	D	Q	860
861	N	P	Q	L	Q	W	N	T	T	E	P	S	R	T	C	K	D	P	I	E	880
881	D	I	N	S	P	E	H	I	Q	R	R	L	S	L	Q	L	P	I	L	H	900
901	H	A	Y	L	P	S	I	G	G	V	D	A	S	C	V	S	P	C	V	S	920
921	P	T	A	S	P	R	H	R	H	V	P	P	S	F	R	V	M	V	S	G	940
941	L																				941